Q-247: Phenotypic microarray analysis of *Desulfovibrio vulgaris Hildenborough*

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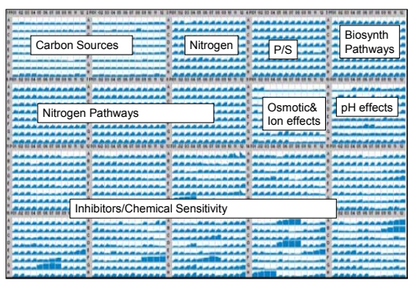
Virtual Institute of Microbial Stress and Survival

Abstract

Phenotypic Microarray™ analysis is a recently developed analytical tool to determine the phenotype of an organism. This technique can be useful to understand the growth changes of an organism when changing medium, temperature, or adding a stressor, or when testing mutant strains. The plates, which are commercially available from Biolog™ (Hayward, CA), consist of array of 20 plates. The first eight plates test a variety of metabolic agents, including electron donors, acceptors, and amino acids. Plates 9 and 10 cover a pH and osmotic stressors, while plates 11-20 contain a variety of inhibitors, including toxic agents and antibiotics. Techniques were developed to use these plates under anaerobic conditions to be able to culture *Desulfovibrio vulgaris*. To accomplish this the plates were set up in an anaerobic chamber and heat sealed in polyethylene bags containing an anaerobic sachet. Using this technique, anaerobic conditions were maintained in the plates for up to a week. Growth of the cells was measured by the increase in turbidity of the cells, which was correlated with both optical densities at 600 nm and total cell counts. Preconditioning of the cells and specialized media preparation are required for the different types of plates in order to get a valid phenotype. The plates have been successfully used to characterize the phenotype of the *Desulfovibrio vulgaris* Hildenborough ATCC 29579 strain and are currently being applied to mutant strains of DvH to provide complete screening of phenotypic changes in knockout mutants, for rapid pathway analyses and modeling.

Biolog Omnilog Phenotype Microarray™ System

•20 X 96 well plates per Phenotype Microarray run for full phenotypic characterization of organism
•Controlled incubation temperature
•Automated imaging and logging at 15 min intervals for more than 200 h
•Omnilog holds 50X 96 well plates at a time



•~2,000 assays per run
•750 metabolic assays
•Carbon Pathways
•P metabolism
•N metabolism
•S metabolism
•Biosynthetic Pathways
•Osmotic and Ion effects
•pH effects
•239 inhibition/sensitivity assays
•Antibiotics: (e.g. Penicillin, amoxicillin)
•Antibacterial (e.g. Sodium azide)
•Toxic ions,(e.g. Bromate, cyanide)
•Toxins (e.g.Atropine, cresol)

1. **PM array of D.Vulgaris, overlaid with targeted systems for each plate.**
2. **Picture of the Omnilog system in our laboratory**
3. **Plates of D.vulgaris. The plates are filled and sealed in bags the Anaerobic chamber for growth in the omnilog**

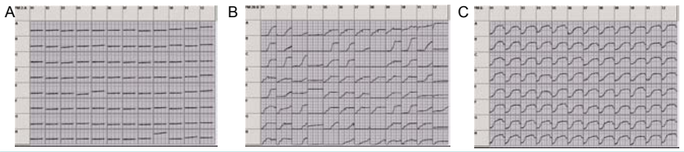
PM Plate Inoculation and Media

Growth of *D.vulgaris* in the 96 well plate format is achieved by starting with a primary culture grown in LS4D medium from a -80ºC stock (composition available by request). When the culture reaches an OD600 of 0.6, usually within 48-72 hours, it is inoculated into the plates using fresh medium at a 10% dilution rate. The plates are then sealed with a heat sealer into Nasco WhirlPak® retain bags, removed from the anaerobic chamber and loaded into the Omnilog system.

Growth in the PM array was accomplished using modified media according to the PM plate type

Plate 1,2 : LS4D without Sodium Lactate
Plate 3,5,6,7,8 : LS4D without Ammonium Chloride
Plate 4 A-E : LS4D without Potassium Phosphate
Plate 4 F-H : LS4D without Sodium Sulfate
Plate 9-20 : regular LS4D

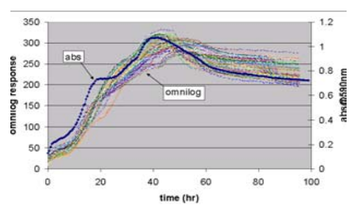
The exact mix of components added to the medium are in the Table below. Antibiotics are added when growing the mutant strains only. Several 96 well plate experiments (blank plates) were completed to optimize the growth of *D.vulgaris* in the plates. One the main issues is carryover of nutrients (e.g. phosphate) in the inoculum allowing the cells to grow independently of the stressor in the PM wells. To overcome this, aliquots of the culture were centrifuged (3000 rpm, 5 minutes), the LS4D removed, and then the cells were resuspended in fresh specialized medium. We also attempted to pre-condition the cell population in specialized media but it was found that it was very difficult to culture *D.vulgaris* in the wells with these stressed and starved cells.

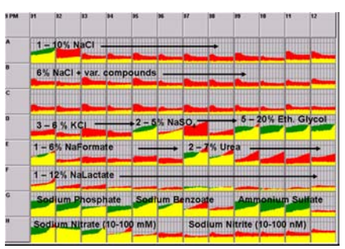


Examples of growth results from the omnilog. Plate A represents underinoculation, in which an insufficient number of viable cells and nutrients were added to support growth. Plate B represents correct inoculation, in which there were enough cells and nutrients to support growth, but growth was affected by the well contents. Plate C represents overinoculation, in which too many cells and nutrients were added, so growth is independent of the well contents.

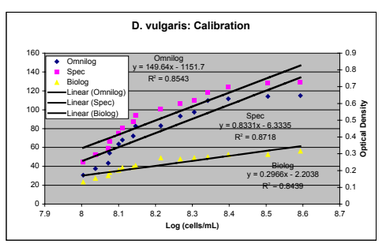
Omnilog Phenotypic microarray D.vulgaris specialized media preparation

LS4D type	ml media	ml culture (centrifuged)	µl. Thaer's vitamins (1:1000 dilution)	µl Titanium citrate (1.2%)	µl G418 (Kanamycin) 50 mg/ml, final concentration 400 ug/ml	µl Ns (Nalidixic acid) 50 mg/ml, final concentration 200 ug/ml
Regular	150	15	150	750	1200	600
No Sodium Lactate	30	3	30	150	240	120
No Ammonium Chloride	60	6	60	300	480	240
No Potassium phosphate	10	1	10	50	80	40
No Sodium sulfate	10	1	10	50	80	40





Example of results for *D.vulgaris* grown on PM 9 which screens for Osmotic and Ion effects.
(Green = mutant, JW7019; Red = wild type; Yellow = overlapping area)



The Omnilog system measures the increase in turbidity of the wells, which was correlated with both optical densities at 600 nm and total cell counts and provided a measure of growth in the plates.

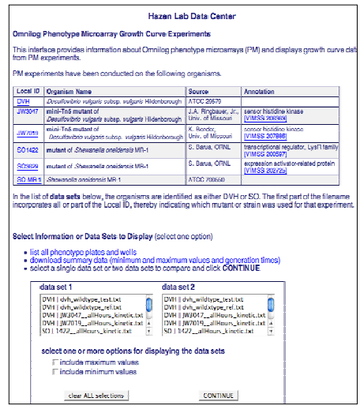
Data Center

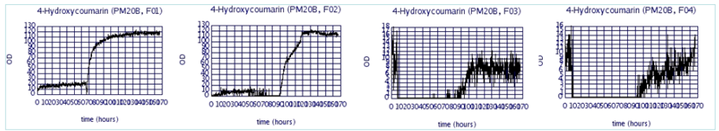
Reviewing Omnilog Phenotype Microarray Growth Curves

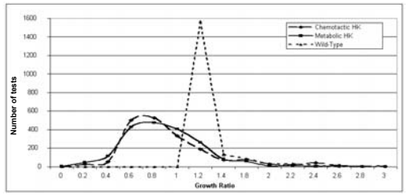
Data analysis and management is challenging for this system, as the Omnilog system creates a large amount of growth data. We have to carefully screen acquired data to ensure that the growth observed is high quality and reproducible.

We have developed a web based data center for viewing and sharing the data. This allows for close inspection and comparisons of maximum values, generation times, and to review data quality.

Below is an example of output from the data center showing the effect of increasing concentrations of 4-Hydroxycoumarin (a rodenticide) on the growth of D.vulgaris. The data shows a clear effect of increased concentrations of this substance on the growth of *D.vulgaris*.







The growth of two histadine kinase mutants, one a chemotactic mutant and the other a generic metabolic mutant, and a wild type replicate were compared to a reference wild type data set by calculating the ratio of growth in each well. The wild type replicate has an average of about 1 with a narrow distribution. The mutants have much different growth pattern, and had less robust growth than the wild type.

Conclusions and Future Work

•To date, we have run approximately 20 Phenotypic Microarray experiments on *D.vulgaris Hildenborough* and mutants.
•We currently have over 50 knockout mutant *D.vulgaris* strains in line for phenotype comparison with the Hildenborough strain.
•Work still needs to be completed on optimization and understanding method variability through analyses of several biological replicates of *D.vulgaris*

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